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The Thyroid and Environmental Stress in Mammals

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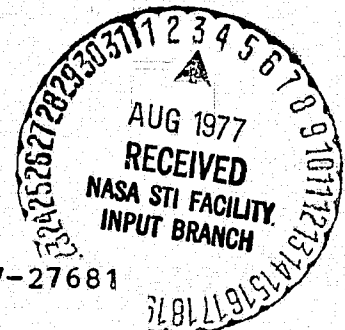
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16. Abstract

The effects of hyperoxia at ambient pressure on thyroid function and thyroid hormone metabolism have been assessed. Thyroidal activity was depressed in mice and rats by exposure to hyperoxia, due at least in part to a decrease in the rate of secretion of pituitary thyrotropin. The effects of hyperoxia on the peripheral deiodination of thyroxine (T_4) were dependent on the concentration of oxygen employed and/or the duration of exposure. When significant changes were observed a reduction in the rate of deiodination and in the deiodinative clearance of T_4 occurred. Hyperoxia also resulted in a marked fall in circulating T_4 concentration and a decrease in T_4 -binding activity in serum. Many of these effects of hyperoxia were prevented by the concomitant administration of large amounts of Vitamin E (α -tocopheryl acetate). These decreases in thyroid function and T_4 metabolism were associated with a decrease in the rate of whole body oxygen consumption.

It was concluded that the deleterious effects of oxygen in the rat were not due, even in part, to an oxygen-induced hyperthyroid state in the peripheral tissues.

Thyroxine was shown to be essential for survival during acute cold stress and but its action was not the result of conversion to triiodothyronine. In large rats, hormone turnover was significantly increased during acute cold exposure. Finally, survival of rats exposed to a simulated altitude of 20,000 feet is greatly improved when the peripheral effects of T_4 are partially blocked by pretreatment with propylthiouracil.

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INTRODUCTION

The role of the thyroid gland in oxygen toxicity

The discovery that high oxygen tensions can kill living tissues was made in 1878 by Paul Bert, who demonstrated that increased oxygen pressures caused convulsions and death in sparrows. For years little attention was paid to the phenomenon of oxygen toxicity. However, as the use of oxygen in medicine, in diving, and in aerospace work increased, the question of the danger of oxygen poisoning became unavoidable. At sea level pressures, 30% oxygen appears to be tolerated for an unlimited period of time. Exposure of both man and animals to 80% oxygen eventually results in mild changes in lung morphology and function, and neurological and neuromuscular symptoms can occur (1,2). However, caution must be used when the effects of increased oxygen pressure are compared in animals and man, or even between different strains of the same species (3). Brooksky, et al. (4) found no evidence of toxic effects during uninterrupted exposure of rats to oxygen at a PO_2 of 450 mm Hg, but dramatic toxic manifestations were seen at 600 and 760 mm Hg. This suggests that a rather definitive threshold exists, at least in rats, for sensitivity to oxygen. This view is supported by others (5), who found that the rat can tolerate sustained exposure to an elevated PO_2 provided the pressure does not exceed 450 - 550 mm Hg. Studies in humans also suggest that there is a tolerance level for man, probably around 450 mm Hg (6,7). Exposure to oxygen at pressures greater than 1 atmosphere can cause severe lung damage (2,7), sterility (1), and ultimately death. Indeed, death can occur in rats exposed to 60 - 80% oxygen at atmospheric pressure (3 - 6). Oxygen poisoning appears to be a time-pressure dependent phenomenon, the two conditions being inversely related. It also appears that the manifestations of oxygen toxicity are greatly influenced by other conditions; for example, associated temperature and humidity, the presence of inert gases, the age of the experimental animals.

In spite of the numerous investigations concerning the etiology of oxygen toxicity (8), the nature of the process is unclear. However, there is little doubt that increased oxygen tension produces alterations in numerous aspects of cellular metabolism and that these metabolic changes eventually lead to a disturbance of cellular function sufficiently great to produce the symptoms of oxygen poisoning seen in the intact organism. Furthermore, cellular changes can be detected before any overt signs of toxicity appear.

While details concerning the primary toxic action (or actions) of oxygen are unknown, two observations were responsible for prompting the investigations described herein. Firstly, the extent of oxygen poisoning is invariably influenced by the rate of oxygen consumption either of the whole body or of a given tissue, and can be influenced appropriately by administration of agents known to alter this rate (9). Secondly, the similarity between the effects of oxygen at high pressure and the effects of excess radiation has led to the view that hydrogen peroxide, or free radicals may be intermediates in the toxic effects of oxygen (10). Studies of peroxidation and oxygen toxicity in biological tissues and the demonstration of a protective effect of antioxidants also provide support for this concept (11).

These two observations are consistent with the possibility that the thyroid gland plays a role in oxygen toxicity, since the thyroid is of major importance in metabolic control, and recent evidence indicates that hydrogen peroxide or free radicals may be involved in thyroid hormone utilization and action in peripheral tissues.

It is well known that the thyroid gland is intimately concerned with the control of basal oxygen consumption and heat production; basal metabolic rate (BMR) varies directly with thyroid status (i.e. the rate of secretion of thyroid hormone by the thyroid gland). Although details concerning the actual site and mechanism of action of thyroxine (T_4) are unknown, from the work of this investigator and others, it is evident that there is a close, although as yet undefined, relationship between the metabolism of T_4 (deiodination) and its physiological action; an increase in the rate of deiodination of T_4 is almost invariably associated with an increase in the effectiveness of the hormone and vice-versa (12).

Other studies in this laboratory have suggested that the physiological deiodination of T_4 is mediated by an H_2O_2 - peroxidase system; deiodination of T_4 is increased in situations in which the peroxide content of the tissue is increased and the process is invariably inhibited by catalase, an enzyme which destroys tissue peroxides (12). If T_4 deiodination is indeed mediated by an H_2O_2 - peroxidase system and the metabolism and physiological action of the hormones are linked, then it follows that both processes will be influenced by conditions which alter the peroxide content of the tissues.

As indicated above, the peroxide content of the tissues may be increased under conditions of hyperoxia. Thus, an associated feature of oxygen toxicity may be an increase in the rate of T_4 metabolism and hence action. This would result in an increase in cellular oxidative metabolism, possibly into the thyrotoxic range. It is of considerable interest, therefore, that the antioxidant, vitamin E, which is beneficial in oxygen poisoning (13) has also been shown to inhibit the rate of deiodination of T_4 in vitro (14).

There is considerable evidence that the extent of oxygen poisoning is indeed influenced by the thyroid status of the animal. The toxicity is potentiated in hyperthyroidism and diminished in hypothyroid (or hypophysectomized) animals (15,16). These differences are thought to be due to the concomitant alteration in metabolic status of the animals; it has been suggested that the rate of CO_2 production and accumulation (which is increased in hyperthyroidism) is an important factor in oxygen toxicity (16,17). However these data do not indicate whether any of the deleterious effects of oxygen are the result of an oxygen-induced increase in T_4 action and metabolism; clearly, any alteration in metabolism could be an effect of O_2 poisoning unrelated to thyroid hormone action.

The present investigation was performed to determine if oxygen poisoning is due in part to an oxygen-induced "thyrotoxic" state in peripheral tissues. The effects of hyperoxia on the rates of thyroidal secretion and peripheral metabolism of thyroid hormone have been assessed, and related to the metabolic status of the animal.

The role of the thyroid gland in acute cold stress

As indicated above, the basal (i.e. resting) metabolic rate is influenced by changes in thyroid status. Oxygen consumption is increased by administration of T_4 ; this increase can be achieved with doses of T_4 only 10-20% greater than normal. However, regardless of the size of the dose, a latent period of 1-3 days always occurs before any changes can be detected. Similarly, following thyroidectomy, at least 24 hrs elapse before any effects of T_4 -deficiency are evident (18).

Survival during sudden cold stress in mammals is dependent on maintaining the rate of heat production equal to the rate of heat loss in order to prevent a fall in body temperature. Since there is only a limited range of temperature in which adequate acute adjustments in the rate of heat loss can be made, it is the rate of heat production that must generally be increased. This is achieved initially by an increase in muscular exercise (i.e. shivering). After some days the animal adapts to the cold and exhibits an increase in resting oxygen consumption known as "non-shivering thermogenesis". In view of the known relationship between the thyroid gland and basal oxygen consumption, and the fact that thyroid function has been shown to be increased in cold-exposed animals (19), it has generally been assumed that T_4 is an important factor in the process of cold-adaptation. However, from studies in this laboratory and elsewhere (19,20) it appears that the "non-shivering thermogenesis" characteristic of the cold-adapted animal is a phenomenon in which T_4 is minimally involved and removal of the thyroid gland in the fully adapted animal has relatively little effect. In contrast, T_4 is essential for survival during the initial phases of cold stress and is necessary for the development of the adapted state (19). A detailed investigation into thyroid function and T_4 metabolism in the cold-adapted animals has already been performed in this laboratory (20). The present report is concerned with T_4 metabolism during the initial phases of cold stress when T_4 is thought to be important. In addition, a study was performed to determine if the survival of rats during acute cold exposure is due to the T_4 per se or whether it follows upon its conversion to 3,3,5'-triiodothyronine (T_3).

Thyroid function and hypoxia

When "normal" rats are suddenly exposed to a reduced atmospheric pressure equivalent of 18,000 feet, 70-80% survive. Death, if it occurs, does so within the first 24 hrs. The survival rate is increased if the rats are thyroidectomized prior to exposure. In fact, at this altitude, group survival is generally complete even if such operated animals are given as much as half the normal daily T_4 requirement (this is a figure that is well established); the rats survive at the altitude indefinitely. At 20,000 feet at least 50% of a group of normal rats do not survive, while again, death only rarely occurs in thyroidectomized rats, i.e., thyroidectomy is beneficial to survival at altitude (19).

In iodine-deficient regions of the world, the common manifestation of the deficiency is goiter (enlarged thyroid gland). In mountainous regions, iodine-deficient goiter is less prevalent at the high elevations than at sea level. This indicates (or at least is consistent with the view) that man is less sensitive to T_4 deficiency at altitude than at sea level.

Stated another way, one requires less T_4 (and hence iodine) to achieve a given effect at altitude than at sea level (19). Conversely, it has been shown in this laboratory that hypoxic stress results in marked decreases in many aspects of thyroid function and T_4 metabolism (21).

One of the major problems which arises on acute exposure to high altitude is hypoxia in peripheral tissues. In the resting state, the rate of oxygen consumption is not normally determined by the supply; supply is adjusted to meet the need. At sea level, within very wide limits, supply can meet the demand. As the atmospheric pressure, and hence the oxygen pressure, decreases, the rate of supply does become limiting, and at high elevations it can become a major problem. Marked physiological changes occur if exposure is continued, but considerable inconvenience and discomfort are experienced during the early phases before acclimatization takes place. Ultimately the body adjusts itself to the point where the tissues are no longer hypoxic. This stage cannot be achieved without delay, and hence, since the supply of oxygen cannot immediately be restored, another approach might be to reduce the demand for oxygen in the tissues.

The rate of oxygen consumption is set at least in part by the systemic concentration of T_4 . The rate of turnover of T_4 is relatively slow (in man the half-life of $^{131}\text{I}-T_4$ is 6-8 days); there is no mechanism for rapidly eliminating T_4 from the system. At altitude, to compound the problem, considerable dehydration occurs in many species, including man, during the initial phase of exposure. In both man and rats this results in an increase in the concentration (not the absolute amount) of T_4 in the system. It is the concentration of T_4 that is the physiologically important factor. Thus, although the latter phenomenon (increased concentration) will prevent further release of T_4 from the gland, for a few days the body has to contend with a normal or even increased concentration of a calorogenic hormone at a time when, ideally, a decrease in peripheral oxygen consumption is necessary.

Since it is not practical to reduce the concentration of T_4 in the system, another possibility in altitude-exposed subjects is to prevent, at least in part, T_4 action. This approach is realistic. Compounds are available which can interfere with both the calorogenic action of T_4 and also the deiodination of the hormone. These factors can be assessed simply from a quantitative standpoint in both man and animals. (Hormonal action and hormonal deiodination are probably associated.) These compounds, which include thiouracil and its propyl derivative, propylthiouracil (PTU), are well-known antithyroid compounds and have been employed for 40 years in the treatment of hyperthyroidism. Their effects on the peripheral action of T_4 have only recently been realized. If indeed one of the major problems is the development of essentially an acute "hyperthyroidism" due to the increased sensitivity of the tissues to the hormone, and/or the inability to remove T_4 from the system during the acute period of peripheral hypoxia, then administration of thiouracil before exposure, thus inducing a decrease in the calorogenic action of the hormone, should prove highly beneficial at altitude.

The present report is concerned with the effect of pretreatment with PTU on the survival of rats at a simulated altitude of 20,000 feet.

METHODS AND MATERIALS
STUDIES ON OXYGEN TOXICITY

Except when stated otherwise, all experiments were performed on male Sprague-Dawley rats weighing approximately 120 g at the start of each experiment and fed Purina Labena rat chow and water ad lib. All rats were maintained at 22° C, and in all experiments a minimum of six rats/group was employed. Rats were subjected to hyperoxia in a large hyperbaric chamber, volume 22 cubic feet which permits the control of both the pressure and the nature of the inflowing gases. Hyperoxic conditions at local atmospheric pressure were achieved by supplying either 100% oxygen or a mixture of oxygen and nitrogen in known proportions to the chamber. Control rats for these experiments were maintained in a comparable but smaller chamber (15 cubic feet) supplied with air at local atmospheric pressure. Chambers were opened for a short period at least every second day for cleaning and feeding. After being open, the chambers were flushed with the appropriate gas at a high flow rate for 1-2 hours. Thereafter the flow rate was reduced but maintained at a rate sufficient to prevent accumulation of significant amounts of water in the chamber. A maximum of six animals per chamber was employed.

Determination of the activity of thyroid gland. Male Swiss-Webster mice (approximately 30 g) were used in these experiments. In each experiment, half were exposed to an hyperoxic atmosphere for a specific period of time, the remainder were supplied with air. Four hours before the end of the exposure period all mice received an ip injection of 1 μ c 125 I-iodide (carrier-free NaI, New England Nuclear Corp.) given in 0.1 ml saline. Four hours later, the mice were killed by a blow and their thyroids quickly removed, placed in a counting tube and their radioactive content determined in an automatic gamma counter (Searle Analytic Inc. Model 1195). The results, expressed either as cpm/thyroid/4 hr or as % dose injected/thyroid/4 hr, were analyzed statistically using methods described by Snedecor and Cochran (22).

Determination of thyrotropin concentration in serum. Two groups of rats were employed. Rats in one group were exposed to 80% oxygen for 48 hr; rats in the other received ambient air. After 44 hr all animals received a single injection of carrier-free 125 I (0.1 μ c/rat). After 48 hr the rats were killed by exsanguination under ether anesthesia and serum and thyroids were obtained. 125 I in the thyroid was determined and expressed as % dose 125 I injected. Thyrotropin (TSH) levels in serum were determined by radioimmunoassay using materials supplied through the courtesy of the National Institutes of Health (NIAMDD) pituitary program.

Measurement of peripheral metabolism of exogenous T_4 . Rats were brought to isotopic equilibrium with a daily subcutaneous dose of 125 I- T_4 of known specific activity, 2 μ g/100 g body weight/day. This dose is considered approximately equal to the amount of hormone normally secreted by the rat thyroid, since it just suppresses hormonal secretion by the thyroid and is sufficient to achieve normal plasma T_4 levels. Thyroidal uptake of any 125 I released from the 125 I- T_4 was prevented by giving 1% NaClO₄ in place of drinking water. (NaClO₄ per se does not significantly alter the peripheral metabolism of T_4). The metabolism of T_4 was assessed as follows. The animals were placed in special individual metabolism cages which permitted the separate collection of urine and feces. Serial 24-hr collections of

urine were made and a sample of tail blood was obtained at the end of each collection period. The amount of ^{125}I in urine was determined by counting aliquots in the counter. Iodide was the only labelled compound detected, either chromatographically or electrophoretically, in urine. The amount of organic ^{125}I in serum was determined as follows: organic and inorganic ^{125}I were separated by short-term paper electrophoresis (90 min) in 0.05 M glycine-acetate buffer, pH 8.6 using a constant current of 12 milliamps. NaI was added as a marker. Under these conditions, the iodide, located by staining with palladium chloride, moved several cm from the origin; the organic iodine remained at the origin. The appropriate sections of the strips were cut and the radioactivity determined in the counter. Chromatographic analysis of the serum in a butanol-dioxane-2N NH_4OH system (4:1:5) revealed that at least 99% of the organic ^{125}I was in the form of T_4 . From these values and the known specific activity of the injected $^{125}\text{I}-\text{T}_4$, the absolute concentration of T_4 in the serum and the quantity of hormone deiodinated (as estimated from the ^{125}I excreted in the urine) were calculated. The data were then used to calculate the rate of clearance of $^{125}\text{I}-\text{T}_4$ from plasma by the deiodinative pathway [i.e. as iodide in urine (23)]:

$$\text{deiodinative clearance} = \frac{\text{urinary } ^{125}\text{I} (\% ^{125}\text{I} \text{ injected as } \text{T}_4/\text{day})/24 \text{ hr}}{(\text{ml plasma}/24 \text{ hr}) \text{ plasma } ^{125}\text{I}-\text{T}_4 (\% ^{125}\text{I} \text{ injected as } \text{T}_4/\text{day})/\text{ml}}$$

With this technique, specific activity equilibrium (as indicated by a constant rate of excretion of ^{125}I in urine) is achieved in 10-14 days. At this point hyperoxic stress was applied. In a few initial experiments, the animals were placed in the chambers in their metabolism cages so that collections could be made during the period of hyperoxic exposure. This proved impractical as only four cages could be inserted together. Thus in the experiments reported here, the animals were exposed to oxygen in regular cages. They continued to receive the daily T_4 injection, and T_4 metabolism was determined during the 24-hr period immediately following the exposure.

Measurement of rate of turnover of T_4 following a single injection of $^{125}\text{I}-\text{T}_4$. The technique using rats equilibrated with exogenous $^{125}\text{I}-\text{T}_4$ has limitations; although the absolute amount of hormone metabolized can be measured precisely, the daily dose has to be selected, it is given in a single daily injection, and no allowance is made for any change in thyroid secretion rate under altered experimental conditions (i.e. hyperoxia). Thus peripheral turnover of T_4 was also determined by the second technique. In this technique, widely used for assessing T_4 turnover in man, endogenous T_4 is labelled with tracer $^{125}\text{I}-\text{T}_4$ and the kinetics of T_4 turnover determined.

The measurement requires three days so this period was included in the total period of hyperoxic exposure. Rats were given an IV injection of a tracer dose of $^{125}\text{I}-\text{T}_4$ (less than 0.01% of total body content of T_4) at 4.0 pm on the first of the days, together with 1 mg NaI in 0.1 ml saline, to prevent ^{125}I uptake by the thyroid. Tail blood samples were obtained at 8.0 am and 8.0 pm on the next two days. (This allowed 16 hr for the tracer to equilibrate with the extrathyroidal endogenous T_4). The NaI injection was repeated on each of these days and the animals continued to receive the appropriate gaseous mixtures. After the last blood sample, the animals were exsanguinated under ether anesthesia. Serum was obtained and the T_4 content determined by the local radioimmunoassay laboratory (courtesy of Dr. Truls Brink-Johnson). Liver, kidney and muscle tissue were taken for

the studies of deiodination in vitro described below. The $^{125}\text{I}-\text{T}_4$ content of sera from the tail blood samples was determined by the electrophoretic technique described above and the percentage of the injected $^{125}\text{I}-\text{T}_4$ in each sample was calculated. For each rat, the rate of disappearance of $^{125}\text{I}-\text{T}_4$ from the serum was plotted on a semi-log paper. After the injected $^{125}\text{I}-\text{T}_4$ is equilibrated with the endogenous T_4 , a straight line can be obtained using the method of least squares analysis. The theoretical plasma concentration of $^{125}\text{I}-\text{T}_4$ at 0 time is determined by extrapolating this line to 0. The slope of the line is the fractional disappearance rate of T_4 (k), the reciprocal of the 0 time extrapolate ($100/\%$ dose/ml) is the total distribution space (TDS). The metabolic clearance rate (MC) = $k \times \text{TDS}$. These turnover studies were carried out in the metabolism cages and both urine and feces were collected. The urinary radioactivity was determined as described above. Feces were homogenized in a mixture of human plasma (to bind any free T_4) and water (1:4) to yield a total volume of 60 ml. Protein bound ^{125}I in feces (which was shown chromatographically to consist entirely of $^{125}\text{I}-\text{T}_4$) was determined by the standard technique of trichloroacetic acid precipitation (20). Urinary and fecal clearance rates were calculated using the mean of the four values obtained for serum $^{125}\text{I}-\text{T}_4$ concentration ($\%$ dose/ml) in each rat.

Assessment of T_4 deiodination in vitro: In order to determine whether changes in the rate of deiodination of T_4 in vivo reflected a change in the intrinsic activity of the deiodinating enzyme rather than an alteration in the intensity of T_4 -binding to protein in serum and extravascular fluids, the rate of T_4 deiodination by isolated tissues in vitro was determined. Homogenates of liver, kidney and muscle from the control and treated rats from the in vivo studies were made in Krebs-Ringer phosphate buffer, pH 7.0, containing 2 mg/ml glucose (KRPB). T_4 -deiodinating activity was measured by incubating the tissue homogenates (1 ml, 1:100 wt/vol) with radioactive T_4 (10^{-9}M) for known periods of time. $^{125}\text{I}-\text{T}_4$ (Amersham-Searle) comes in 50% propylene glycol and appropriate dilutions in 0.01% human serum albumin were made for in vitro studies. At the end of incubation the reaction was stopped by addition of human plasma (0.3 ml), which binds any available hormone and prevents further metabolism. The percentage of organic and inorganic ^{125}I in the reaction mixture was determined by the electrophoretic technique described above. The ^{125}I -labelled products formed were also determined in some experiments by chromatographic analysis. T_4 and inorganic iodide were the only labelled compounds present in significant amounts. The percentage of the $^{125}\text{I}-\text{T}_4$ deiodinated by a tissue preparation was corrected for iodide generated non-enzymically in tissue-free control incubations, or in incubations carried out with pre-boiled tissue.

Determination of serum T_4 -binding activity. Serum T_4 -binding activity was assessed using a charcoal-binding assay technique developed in this laboratory. Each serum sample was diluted 1:500 with Krebs-Ringer phosphate buffer containing 2 mg/ml glucose (KRPB). 1 ml aliquots were incubated with $^{125}\text{I}-\text{T}_4$ (added in 10 μl to yield 10^{-8}M) for 30 min at 37°C . The percentage of labelled hormone bound to tissue components was determined by mixing an aliquot of labelled serum (100 μl) with 100 μl charcoal solution (1.0 g Norit Neutral and 0.1 g Dextran 60 in 100 ml 1.5 mM MgCl_2) prechilled to 4°C in a 1.5 ml capped plastic tube (Eppendorf). The mixture was shaken on a vortex agitator for 5 sec, held at 4°C for 60 min

and then centrifuged at 4° C in an Eppendorf centrifuge (Model 3200) for 2 min. The proportion of organic to inorganic ^{125}I in the supernatant was determined electrophoretically as described above. The total counts and their distribution between organic and inorganic iodide in the serum mixture were determined by revortexing a 1:1 mixture of charcoal solution and labelled serum after holding at 4° C for 60 min, electrophoresing and counting. The ^{125}I - T_4 remaining in the supernatant represented that tightly bound to tissue protein. Relative binding activity was thus calculated as the percentage of the total ^{125}I - T_4 in the supernatant.

Measurement of oxygen consumption. Oxygen consumption was determined in rats exposed to 60% and 100% oxygen. Measurements were made before exposure and on each day of exposure in an apparatus purchased from Medical Science Electronics, Inc., St. Louis, Missouri. It consists of a small plastic chamber attached to a volume meter (Model 160). The chamber was maintained at a relatively constant temperature by a surrounding water jacket. Soda lime (Fisher Scientific Co.) and Drierite (Hammond Drierite Co., Xenia, Ohio) were placed in the chamber to absorb the exhaled CO_2 and water vapor. The measurements were made after flushing the chamber with 100% oxygen. Oxygen uptake is recorded on graph paper by the pen mechanism of the volume meter; the pen moves horizontally at a rate reflecting the rate of oxygen uptake in the chamber. The pen also makes a vertical movement every minute. A horizontal movement of 1cm is equal to 0.673 ml oxygen. Thus oxygen uptake can be calculated. For each rat, measurements were made for 30-60 min, thus allowing the rat to settle down. Because of the stressful nature of the experiment, the animals were not food-restricted and thus the measurement cannot be considered a basal metabolic rate. Values given are the mean of 30-60 min of observations and are expressed as oxygen consumed/minute/rat.

STUDIES ON ACUTE COLD STRESS

All studies were performed on adult male Sprague-Dawley rats weighing approximately 200 g at the start of an experiment.

Measurement of T_4 metabolism in vivo. Rats were brought to isotopic equilibrium with ^{125}I - T_4 and the in vivo metabolism of T_4 over a 24 hr period assessed as described above. Half the rats were then placed in a cold room (4° C), the remainder continued to be maintained at ambient room temperature (22°). Serial 24-hr measurements of T_4 metabolism were made for 4 days.

Determination of turnover of endogenous T_4 . Two groups of rats were employed per experiment. One group was placed in the cold room. The other was kept at room temperature. After the cold-exposed group had been in the cold for 16 hr, all rats in both groups received an iv injection of a tracer dose of ^{125}I - T_4 and turnover was measured over the next three days as described for the oxygen toxicity studies.

Assessment of the role of deiodination in combating acute cold stress. Three types of experiments were performed. In the first, four groups of animals were employed: 1) rats were fed powdered Purina Labina rat chow; 2) rats were fed the chow supplemented with PTU (2mg/g food); 3) rats were fed powdered diet and received a daily sc injection of T_4 (2ug/100g BW);

4) rats were fed PTU diet and were also given a daily injection of T_4 (2ug). After three weeks on this regimen they were all placed in the cold room and the % survival of rats in each group was observed.

In the second series of experiments, the effects of both PTU and cold exposure on the metabolism of exogenous T_4 were determined. Twenty-four rats previously brought to approximate $^{125}I/^{127}I$ isotopic equilibrium by administration of $^{125}I-T_4$ (2 $\mu g/100$ g BW/day) were employed. Thyroidal uptake of ^{125}I was prevented by $NaClO_4$, given as a 1% solution in the drinking water. On day 1, the equilibrated rats were divided into four groups and were placed in individual metabolism cages. Rats in two groups were fed powdered Purina Labena rat chow and rats in the other two groups were fed the same diet containing PTU (2 mg/g food). Rats from one group on each diet were maintained at 22° C throughout. The remainder were exposed to 4° C from day 11 to day 14 (the end of the experiment). The $^{125}I-T_4$ and $NaClO_4$ were given throughout. The animals were weighed daily and on days 8, 13, and 14, 24-hr urine collections were made. On the first day of exposure one collection was made over the first 8-hr period (designated as day 11) and a second over the subsequent 16-hr period (day 12). Tail blood samples were obtained at the end of each collection period. From the ^{125}I counts in serum, urine and an aliquot of the $^{125}I-T_4$ solution used for injection, the urinary excretion of iodide derived from T_4 , the serum concentration of T_4 and the urinary clearance of T_4 iodine (deiodinative clearance) were calculated as described above).

In the third type of experiment the production of T_3 from a maintenance dose of T_4 in PTU-treated, cold-exposed rats was determined. Four groups of rats were treated in the following ways: 1) rats were fed unsupplemented powdered diet; 2) rats were fed PTU-diet (2 mg/g food); 3) rats were fed unsupplemented diet and were given a daily sc injection of T_4 (2 or 4 $\mu g/100$ g BW); 4) rats were fed PTU-diet and were given T_4 (2 or 4 $\mu g/100$ g BW/day). The diets were given for 2-4 wks and the T_4 was administered for the last 4 days prior to cold exposure. The diets and T_4 were continued during cold exposure. At the end of 3 days of cold exposure all animals were exsanguinated under ether anesthesia. Since rats in Group 2 did not survive 3 days at 4° C, blood was obtained from extra rats maintained on the PTU diet but not exposed to cold. Serum T_4 and T_3 levels were determined by radioimmunoassay. (Radioimmunoassay kits for T_4 , tetramuno, and T_3 , trimuno, were purchased from the Curtis Nuclear Company, 46th Street, Los Angeles, California 90058.)

STUDIES CONCERNING T_4 METABOLISM AND HYPOXIA

Experiments were performed to determine whether survival of rats acutely exposed to a simulated altitude of 20,000 ft could be enhanced by partially inhibiting the peripheral deiodination of T_4 . Two groups of rats were employed in each experiment (10 rats/group). All rats in both groups received 1% $NaClO_4$ in the drinking water to minimize endogenous thyroid function and were maintained with a daily injection of T_4 (2 $\mu g/100$ BW/day). After ten days on this regimen, rats in one group received PTU diet (2mg/g), rats in the other group received regular powdered chow. Three days later all rats were placed in the hyperbaric chambers and the internal pressure was reduced to simulate an altitude of 20,000 ft. The % survival of rats in each group was observed.

RESULTS

Studies of oxygen toxicity. During exposure to O_2 (40-100%), the rats did not appear to be under an obvious physical strain comparable to that which occurs during exposure to altitude (18,000 ft) or cold $4^\circ C$ (23,24). At 40-60% oxygen no significant changes in food and water intake or body weight were observed. At 80% oxygen, there was some decrease in food intake and body weight during the first 24-hr period but thereafter normal food consumption was resumed at least for 5 days, (the maximum period of exposure to 80% oxygen employed). With 100% oxygen, food consumption was reduced during the first 24 hr, but improved thereafter. However, 150 g rats were unable to survive in a 100% oxygen atmosphere for 5 days.

Exposure of mice to increased oxygen concentrations at atmospheric pressure resulted in a decrease in thyroid function, as indicated by a decrease in the rate of uptake of ^{125}I by the thyroid gland (Table 1). Thyroid uptake was measured over a 4-hr period to minimize the error introduced when newly synthesized labelled ^{125}I - T_4 begins to be secreted in significant amounts from the gland (after 8 hrs). The effect of oxygen was dependent on both the concentration of the gas in the mixture and the duration of exposure. With 100% oxygen, thyroid function was depressed within 24 hrs. With 80% oxygen, a significant depression was generally not observed until 48 hrs. When 40% oxygen was administered, an effect was not consistently observed until after 72 hrs.

The decrease in thyroid function which occurred following exposure to hyperoxia could be prevented to some extent by administration of Vitamin E. Vitamin E was administered either as a sc injection of Vitamin E in oil (20 mg/rat); controls received an equivalent amount of vehicle, or in the food (20 mg α -tocopheryl sulphate/10 g food). In a series of experiments, it was found that Vitamin E was ineffective when given only during the period of hyperoxic exposure. However, as shown in Figure 1, if Vitamin E was given in the diet for 3 days prior to oxygen exposure and in addition, injected on each of the days of exposure, the depression of thyroid function seen in the control mice exposed to oxygen was prevented.

The decrease in thyroid function was due at least in part to a decrease in circulating levels of pituitary TSH. The data shown in Table 2 illustrate the concomitant decreases in thyroidal activity and serum TSH levels in rats exposed to 80% oxygen for 48 hr.

The effects of oxygen exposure on the metabolism of T_4 in rats isotopically equilibrated with ^{125}I - T_4 are shown in Table 3. Exposure to 40-80% oxygen for 96 hrs generally resulted in a decrease in the peripheral metabolism of T_4 . This was manifested in a decrease in the amount of iodide derived from T_4 excreted in urine and usually this decrease was associated with a significant increase in the concentration of T_4 in serum. These values were used to calculate the urinary or deiodinative clearance which was almost invariably decreased.

Some studies were performed with 100% oxygen. In these, there was an indication that T_4 metabolism was decreased within 36-48 hrs. However, these experiments were not satisfactory because a decrease in food intake occurred during the first 24 hrs of exposure to 100% oxygen. This

phenomenon per se has been shown to alter T_4 metabolism (24). Attempts to pair-feed were largely unsuccessful due to animal variation. In the experiments illustrated in Table 3, all rats consumed the same quantity of food during the 24 hr collection period.

The effects of hyperoxia on the rate of turnover of endogenous T_4 are shown in Table 4. In these experiments, rats exposed to 80-100% oxygen were at least 250 g; they withstood the oxygen stress much better and were more suitable for the repetitive bleedings than were the small rats (150 g). Hyperoxia for 3-7 days had no consistent effect on the fractional rate of turnover of $^{125}\text{I}-T_4$ or the total distribution space. However, the exposure invariably resulted in a marked decrease in the concentration of T_4 in serum. Thus the absolute amount of hormone utilized in the hyperoxic rats was reduced. It should be noted that in these experiments the thyroid gland was functioning and the decrease in serum T_4 (unlabelled) was presumably due in part to a decrease in the rate of T_4 secretion from the thyroid. In the animals equilibrated with $^{125}\text{I}-T_4$, no provision was made to alter the daily supply of T_4 to simulate this decrease, and thus the serum T_4 levels were generally elevated.

As shown in Table 4, the calculated total metabolic clearance was rarely altered in the hyperoxic rats. However, changes were generally observed in the urinary and fecal clearance calculated from the measurements made of urinary ^{125}I and fecal $^{125}\text{I}-T_4$. Urinary clearance was decreased while fecal clearance of T_4 was increased. Again, since the serum T_4 levels were reduced in the hyperoxic rats, these data indicate that the absolute amount of T_4 removed from the plasma by the deiodinative pathway per unit time was significantly decreased.

The decrease in deiodination of T_4 observed in rats exposed to hyperoxia could, under certain conditions, be prevented by administration of Vitamin E. Administration of Vitamin E only during hyperoxic exposure was insufficient. However, if Vitamin E was administered in the food for 3 days prior to exposure and in addition was injected during exposure, the decrease in T_4 metabolism resulting from the hyperoxia became insignificant (Table 5).

In order to determine whether the decrease in hormonal deiodination in vivo reflected an actual decrease in the intrinsic deiodinating activity of peripheral tissues or was due merely to an alteration in the rate of transport of the hormone to the tissues, the T_4 -deiodinating activity of liver, kidney and muscle was determined in vitro. As shown in Table 6, the deiodinating activity in homogenates of muscle (1:100 w/v) prepared from rats exposed to 80% O_2 for 48 hrs was significantly decreased. In contrast, activity in both liver and kidney were unaffected by the exposure.

One of the major factors influencing peripheral metabolism of T_4 is the intensity of binding of the hormone to serum proteins. A change in serum binding activity is generally associated with a change in the rate of metabolism of the hormone. A significant decrease in the serum T_4 -binding activity occurred in rats exposed to 80% oxygen for 48 hrs. The changes in serum binding activity were not apparent after only 24 hrs of exposure (Table 7).

Effect of hyperoxia on oxygen consumption. Studies were made on rats exposed to 60% oxygen and 100% oxygen. With 60% oxygen exposure, measurements were made on each of the five days of exposure. Although, the means of values obtained in the exposed rats on days 4 and 5 were invariably lower than that of the controls, the data were not statistically significant. Similar findings were made in rats exposed to 80% oxygen for 1 day. However, after 48 hrs in 80% oxygen, oxygen consumption was significantly depressed (Table 8).

Studies on acute cold stress.

The effects of acute exposure of rats to 4° C on the metabolism of exogenous $^{125}\text{I}-\text{T}_4$ are shown in Table 9. Cold exposure resulted in a significant loss of body weight, even during the first 24 hr. However, at no time was the rate of deiodination of T_4 (measured as iodide in urine) enhanced. In fact on two of the days a significant decrease occurred. The only other effect was an inconsistent increase in the calculated fecal clearance of T_4 .

It is evident that cold exposure imposes a considerable stress on these animals, indicated in part by the loss of body weight. As is discussed below in detail the method used to determine T_4 metabolism in vivo has its limitations, one of which relates to the dose of hormone administered. In this case rats received 2 ug/100 g BW/day based on the initial weight of the rat. Since cold-exposed rats lost weight they received more hormone per unit weight during exposure than the control rats. Furthermore, the exposure results in an enhanced rate of secretion of hormone from the thyroid gland (19). To circumvent some of these problems, the turnover of endogenous T_4 was measured also using the method which employs a single injection of tracer $^{125}\text{I}-\text{T}_4$. The results are shown in Table 10. These data were obtained in rats weighing more than 300 g at the start of the experiment. When animals weighing less than 200 g were employed, changes in T_4 kinetics were only rarely and inconsistently observed. (This may be related in part to the loss of weight which was more marked in the younger animals.) When the large animals were exposed to cold, significant increases in the metabolic clearance and deiodination of T_4 (as indicated by % dose ^{125}I in urine) were observed. Furthermore the biological half-life of the injected T_4 was significantly decreased in cold exposed rats.

The importance of thyroid hormone for survival during acute cold exposure is illustrated by the results shown in Figure 2. Rats pretreated for 3 weeks with PTU (which prevents synthesis of T_4 and T_3) were totally unable to survive cold exposure. (In experiment 1, PTU was given for only 2 weeks and 1 animal survived, presumably due to the presence of residual T_4 .) However, death was generally prevented by administration of T_4 to the PTU treated rats (Fig. 2).

The effects of PTU and cold on the deiodination of T_4 are shown in Fig. 3. After 8 days on PTU but prior to cold exposure, urinary excretion of iodide derived from T_4 was reduced by two-thirds in rats receiving PTU. No further changes were observed in rats on either diet during the first 8 hr of exposure to 4° C and no changes were observed in rats on regular chow during the entire 3-day exposure. In contrast, deiodination

was significantly enhanced in the PTU-treated rats during the latter 16 hr of the first day of exposure (day 12) and during day 13 although the value was still less than 50% of that obtained in cold-exposed rats fed regular diet. By the third day of exposure (day 14) deiodination in the PTU-treated rats was not significantly different from PTU-fed rats kept at 22° C. In these experiments, differences in food intake between rats undergoing the various regimens were too small to have contributed significantly to any changes in the rate of deiodination of T_4 .

The effects of cold and PTU on serum T_4 levels and urinary clearance of T_4 from plasma (deiodinative clearance) are shown in Fig. 4. When measured before exposure to cold, serum T_4 levels were somewhat elevated in PTU-treated rats. During cold exposure, T_4 levels in rats fed regular food were rarely significantly altered, but a marked decrease in serum T_4 concentration invariably occurred in rats given PTU. Deiodinative clearance in rats fed regular food was unchanged in the cold. However, the observed increase in urinary excretion of iodide derived from T_4 in PTU-treated rats was reflected in a significant increase in the calculated deiodinative clearance.

Serum T_4 and T_3 concentrations, measured by radioimmunoassay, in these rats are shown in Fig. 5. PTU resulted in a marked decrease in the circulating level of T_4 . In experiments 1 and 2, when PTU was given for 2 weeks, detectable amounts of T_4 and T_3 were present in the serum but these concentrations were apparently insufficient for survival. Four weeks on PTU (experiment 3) resulted in values for serum T_3 concentration which were below the value considered significant in the assay employed. Administration of T_4 to rats on either diet resulted in T_4 levels which were close to normal, even when twice the usual dose was given (experiment 3). In the group given T_4 but not PTU, significant amounts of T_3 were present in serum. However, in the presence of PTU, values for serum T_3 were at or below the level of significance.

Studies on T_4 metabolism and hypoxia. The effect of PTU on the survival of rats receiving a maintenance dose of T_4 during exposure to simulated altitude of 20,000 feet is shown in Table 11. At least 50% of rats receiving T_4 but no PTU did not survive this degree of hypoxia. However, survival was greatly improved when the T_4 -treated rats were also pretreated with PTU.

DISCUSSION

The role of the thyroid in oxygen toxicity

The results obtained in these studies clearly indicate that several aspects of thyroid hormone economy are decreased in rats exposed to elevated concentrations of oxygen. The effects were induced by exposure to oxygen concentrations ranging from 40% to 100% at local atmospheric pressure. However, considerably more time was necessary for the effects to be manifest with 40% than with 100% oxygen.

The methods employed for assessing thyroid function and hormonal metabolism in these studies are well established and many have been used in this laboratory for several years. Thus most of the associated problems and

pitfalls are known and appropriate adjustments either in technique or interpretation can be made.

To test for thyroid function, the standard radioactive iodine uptake test was employed. Mice were chosen for this study because relatively large numbers are needed. There is also less variation in iodine uptake in mice than in rats. In the hyperoxic mice thyroïdal uptake of ^{125}I was decreased. The possibility that this was due not to depressed ^{125}I uptake but to an enhanced rate of secretion of newly synthesized $^{125}\text{I}-\text{T}_4$ was virtually excluded by measuring uptake over a 4-hr period. Normally, $^{125}\text{I}-\text{T}_4$ is not present in significant amounts in plasma until at least 8 hrs following injection of ^{125}I . In two of the experiments, absence of organic ^{125}I in plasma of both control and exposed mice was confirmed by electrophoretic analysis.

A second pitfall in this technique relates to the $^{125}\text{I}/^{127}\text{I}$ ratio achieved following injection of the isotope. Provided the endogenous iodide content of animals in each group is comparable, a change in ^{125}I uptake reflects a similar change in uptake of stable iodine (^{127}I). However, if the treatment or experimental conditions induce a marked change in endogenous iodide content, it is possible for the specific activity achieved to be sufficiently different from the controls that a change in radioactive iodide uptake does not reflect a similar change in stable iodide uptake. However, in the present experiments, any changes in food and water intake and excretion were insufficient to warrant concern regarding serious alterations in endogenous iodide levels.

The apparent decrease in thyroid function in hyperoxic animals was invariably associated with a marked decrease in plasma T_4 concentration. Theoretically a decrease in circulating T_4 concentration can result either from a reduction in the rate of secretion of T_4 by the thyroid or from a decrease in the T_4 -binding activity in plasma. In these experiments both phenomena appeared to be involved. Thyroid function as indicated by the ^{125}I -uptake test, was decreased. This was due at least in part to a reduction in the plasma concentration of thyroid-stimulating hormone (TSH). However, by direct measurement it was found that the total T_4 -binding activity in plasma was reduced in exposed animals. It is well known that most of the T_4 circulating in plasma is bound to serum proteins; less than 1% of the total plasma T_4 is in the free form. However, it is the concentration of free T_4 in plasma that under normal conditions is maintained constant. For example, if an alteration in binding activity occurs, the percentage of free T_4 and hence the concentration of free T_4 is changed. Thyroïdal secretion of T_4 must then be altered acutely to restore to normal the concentration of free hormone. At this point a new equilibrium between bound and free T_4 is established and the T_4 secretion rate can resume its original level. In this way a change in binding activity and hence total plasma T_4 concentration can occur without any permanent change in thyroïdal secretion rate or T_4 turnover. In the present experiments, although the change in binding activity must have contributed to the reduction in plasma T_4 levels, it is unlikely that this was entirely responsible for the change. Firstly, the decrease in binding activity was relatively small and was probably insufficient to account entirely for the observed drop in plasma T_4 concentration. Secondly, thyroïdal uptake ^{125}I was decreased and it is therefore reasonable to assume that the rate of release of T_4 by

the gland into the circulation was also decreased. It should also be noted that the fall in plasma T_4 levels was masked to some extent by the decrease in peripheral turnover of the hormone which must have counteracted the change to some extent. Some insight into the relative contribution of the changes in T_4 secretion rate and T_4 -binding activity to the decrease in T_4 concentration could be obtained by determining the concentration of free T_4 in plasma of exposed rats. A decrease in free T_4 concentration would suggest that the pituitary-thyroid axis is unable to respond adequately and restore the free T_4 concentration to its normal value. Unfortunately determination of free T_4 levels in rat plasma is difficult since there is a great deal of animal variation. Such determinations were not made in this study.

The peripheral metabolism of T_4 was assessed by two methods. Both have advantages and disadvantages. The isotopic equilibrium method is useful because it enables the investigator to determine the metabolism of known amount of hormone in the absence of a functioning thyroid gland. Thus unknown variations in endogenous hormone secretion rate are avoided. However, with this method, it is necessary to make a decision concerning the amount of hormone required to simulate the physiological level, a task that is not always easy. Furthermore, the continuous secretion of T_4 by the thyroid cannot be mimicked precisely when hormone is supplied by injection once (or twice) a day. In most cases, however, these problems are not serious and can even be used advantageously. In the present study it was evident that the dose of T_4 administered was on the high side since the serum T_4 levels achieved in the exposed rats were generally increased; in normal rats exposed to oxygen, plasma concentration of endogenous T_4 was decreased. Since it has been shown that in normal rats the amount of hormone deiodinated is proportional both to the dose of T_4 and to the serum concentration of T_4 , when the dose range is between 1 and 6 $\mu\text{g}/100 \text{ g body weight/day}$ (25), the observed decrease in T_4 deiodination and its clearance from plasma in the presence of elevated plasma T_4 levels in rats is particularly convincing.

The second method utilizes the intact rat and was employed primarily to confirm and substantiate the results of the study with exogenous T_4 . The technique provides information regarding many aspects of thyroid hormone economy and the data obtained emphasizes the importance of detailed investigations. For example the total metabolic clearance and the fractional rate of turnover of T_4 were not influenced by hyperoxia. On the surface these findings appear to contradict those obtained in the experiments with endogenous T_4 . However, these measurements reflect the combined turnover or clearance of T_4 by both the urinary (deiodinative) and fecal pathway. The latter represents merely a loss of hormone to the system. When each was determined directly it was evident that urinary clearance was, as in the other experiments, depressed. Since there was a concomitant and comparable increase in fecal clearance, the total clearance was unchanged.

The significant increase in fecal clearance of T_4 was unexpected in view of the decrease in plasma T_4 concentration. This observation suggests that the handling of T_4 by the liver (conjugation and excretion in bile) is in some way influenced directly in the hyperoxic animals.

These tracer turnover studies substantiate the observation in the equilibration studies that the absolute amount of T_4 deiodination was decreased; although the fractional rate of turnover of T_4 was unchanged, the plasma T_4 concentration was decreased, thus the actual amount of T_4 deiodinated must have been reduced. It was therefore concluded from these two studies that the hyperoxia in rats results in a decrease in the rate of deiodination of T_4 in peripheral tissues.

As mentioned above, a change in the rate of peripheral metabolism of T_4 can result from a change in the intrinsic activity of the deiodinating mechanism. It also can be due to an alteration in the rate at which the hormone is transported in the tissues. For example, if plasma T_4 -binding activity is decreased, there follows an increase in the percentage and temporarily also in the absolute amount of free hormone in plasma and this rapidly equilibrates throughout the peripheral tissues. Conversely a decrease in binding activity results in a reduction in the percentage of free T_4 in plasma and hence also in peripheral tissues. In the present work, the decrease in peripheral deiodination of T_4 could not be attributed to an increase in plasma T_4 -binding activity since binding activity was reduced.

The deiodinating activity of several individual tissues was measured in vitro. It appears that the tissue primarily responsible for the decrease in rate of deiodinating is muscle. No significant alteration in activity was observed in liver and kidney.

These findings clearly indicate that the hyperoxic rat is not thyrotoxic; both the action and metabolism of T_4 were depressed in peripheral tissues and thyroid activity was reduced. The decrease in thyroidal activity could not be attributed solely to the decreased turnover of T_4 in peripheral tissue since serum T_4 levels were also decreased. The reduction in circulating T_4 concentration suggests that the pituitary-thyroid system, which normally serves to maintain serum T_4 levels, was not operating adequately under these conditions. However, the decrease in thyroid function could also have resulted from a decrease in the rate of secretion of thyrotropin-releasing hormone from the hypothalamus.

The data also do not support the view that T_4 deiodination is mediated by an H_2O_2 -peroxidase system since, under conditions in which the H_2O_2 content of the tissues has been found to be elevated (10), the rate of deiodination of T_4 was decreased. The decrease, at least in muscle, was intrinsic to the tissue and was not the result of changes in the rate of delivery of hormone to the tissue (indeed in some studies it was found that the concentration of ^{125}I - T_4 in liver and muscle was the same in hyperoxic and control animals). Therefore, the potential for T_4 deiodination to be increased by peroxidase-mediated system was present. The finding that deiodination was actually decreased under these conditions suggests that H_2O_2 has little, if any, role in T_4 deiodination in vivo.

On the other hand, as is almost invariably the case, concomitant changes in T_4 action and T_4 metabolism were observed; both were reduced. Unfortunately, one can conclude little from this other than that the two phenomena may be associated. It is possible that hyperoxia interferes with T_4 deiodination and hence its action. Conversely, since T_4 influences

oxidative processes in the cell and the deiodination of T_4 is also an oxidative process, hyperoxia may interfere primarily with T_4 action, resulting in a secondary alteration in the its of metabolism.

How these effects of hyperoxia are mediated is not clear. It is well known that exposure of man and animals to hyperoxic conditions of the type employed herein results in only small changes in pO_2 in all tissues with the exception of lung. It is difficult to accept that the deiodinative metabolism would be decreased directly by small changes in pO_2 , particularly since the activity of the mechanism, when studied in vitro is increased in an atmosphere of 95% O_2 (26). Evidently the effects of O_2 on deiodination in vivo and in vitro are different. It seems more likely the depression of T_4 deiodination is an indirect effect of O_2 . The possibility cannot be excluded that the peripheral effects of hyperoxia observed in these studies are secondary to changes in the lung, the tissue that exhibits the greatest change in pO_2 .

In summary, it is clear that the deleterious effects of hyperoxia in vivo cannot be attributed, even in part, to an increase in the thyroid function. On the contrary, one of the effects of hyperoxia is to render the rat somewhat hypothyroid.

The role of the thyroid gland in acute cold stress

The data obtained from the studies in the isotopically equilibrated rats are complex and difficult to interpret. When rats are exposed to cold there is a significant loss of weight, marked changes in food intake (an immediate reduction, followed by an increase) and a significant increase in the rate of secretion of hormone from the thyroid (19). Thus the technique of a single daily injection, the amount based on body weight, is not an ideal method for this study. For this reason the turnover of endogenous T_4 was determined by the tracer injection technique. With this method, consistent results were only seen in relatively large animals. In such animals, T_4 turnover was clearly increased. The differences in results from large and small animals may be more apparent than real. Small rats survive less well than large rats in the cold and those that do survive initially exhibit signs of marked stress and discomfort before they finally settle down and resume eating, etc. Partial starvation per se reduces T_4 deiodination (24) and produces other marked changes in T_4 turnover. Thus one may be measuring the result of two stresses, cold stress and food deprivation. The problems are much less evident in large rats since food intake is only slightly decreased and these rats appear to tolerate the cold better than their young counterparts.

The importance of deiodination in the hormonal action of T_4 is unclear. Several investigators have demonstrated that PTU decreases the physiological response to a given dose of T_4 in the rat (27,28) and the observations made in this laboratory indicating that growth is retarded in rats receiving PTU in addition to the maintenance dose of T_4 is consistent with this view. The present results confirm previous reports that PTU decreases the rate of deiodination of exogenous T_4 in rats (29,20) and to this extent they support the possibility that the action and deiodination of T_4 are in some way related. However, the observations made concerning T_4 deiodination during acute cold exposure cast some doubt on this viewpoint.

Rats receiving PTU alone, invariably die during acute exposure to cold unless they are given T_4 . Since unexposed rats on PTU continue to survive, there is clearly a requirement for T_4 in the cold. Yet it was noted that in the ^{125}I - T_4 equilibrated rats not receiving PTU, deiodination was not enhanced during cold exposure. Moreover, although the PTU-treated rats exhibited a significant increase in the rate of deiodination during the first two days of cold exposure, the increase was small, and was not observed after the second day. Thus the importance of deiodination in the action of T_4 in these experiments is still open to question.

There is little doubt that T_4 is converted to T_3 in peripheral tissues of many species including man, and some investigators feel that much, if not all, the metabolic effectiveness of T_4 is obtained only after its monodeiodination to T_3 . The studies with PTU suggest that this is not the case in cold-exposed rats. In the rats treated with PTU alone for 2 weeks, serum T_4 levels were reduced 50% or more. Serum T_3 concentrations were also greatly reduced. When these PTU-treated rats were given a maintenance dose of T_4 they survived exposure to cold. In this case, serum T_4 levels approached normal but serum T_3 concentrations were very low, probably insignificant. Indeed, in two of three experiments, serum T_3 levels were lower in the rats treated with both PTU and T_4 than in those given PTU alone, yet rats in the former group survived while those in the latter did not. These results are consistent with the view that monodeiodination of T_4 to T_3 is not a mandatory step for T_4 action; T_4 has intrinsic biological activity.

T_4 metabolism and hypoxia

The results of this study indicate the PTU is beneficial to survival of rats at a simulated altitude of 20,000 feet. Since control and experimental animals were receiving the same amount of hormone and thyroid function was blocked with NaClO_4 . The effect could not be due to the action of PTU on the function of the thyroid gland per se. The effect must therefore be due to interference by PTU of thyroid hormone action in peripheral tissues, either directly, or possibly through a suppression of peripheral deiodination.

These results are consistent with the observations of others concerning the detrimental effect of thyroid hormone in hypoxia.

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TABLE I

Effect of hyperoxia on thyroid function in mice

Expt.	% oxygen	Hr. exposed	Thyroidal ^{125}I - uptake (cpm/gland/4hr)	Control	Exptl.	p value
1	100	24		$327 \pm 37^+$	238 ± 22	NS
2	100	48		327 ± 37	216 ± 11	< 0.025
3	100	24		252 ± 26	142 ± 14	< 0.001
4	80	24		85 ± 10	61 ± 5	NS
5	80	48		85 ± 10	50 ± 5	< 0.05
6	80	24		1145 ± 65	901 ± 93	< 0.025
7	60	48		1054 ± 189	869 ± 87	NS
6	60	48		1566 ± 62	1060 ± 64	< 0.001
7	60	72		1566 ± 62	1024 ± 72	< 0.001
8	60	48		1451 ± 65	824 ± 75	< 0.001
9	50	48		4170 ± 467	701 ± 239	< 0.001
10	40	72		1481 ± 598	753 ± 50	NS
11	40	72		3631 ± 252	2351 ± 224	< 0.005

+ Mean \pm se

TABLE 2

Effect of hyperoxia on thyroid function and serum
thyrotropin levels in rats

	Thyroidal ^{125}I uptake/4 hr (% ^{125}I injected)	Serum TSH (ng/ml)
Control	5.96 ± 0.47	384 ± 44.5
Hyperoxic	4.68 ± 0.33	263 ± 19.7
p value	< 0.05	

Values are mean \pm se

Hyperoxic animals were exposed to 80% O_2 for 48 hr.

TABLE 3

Effect of hyperoxia on T_4 metabolism in rats isotopically equilibrated with $^{125}I-T_4$

Expt.	% oxygen	Duration of exposure(hr)	Group	Urinary I^- ($\mu g \equiv T_4/24$ hr)	Serum T_4 ($\mu g/100ml$)	Urinary clearance of T_4 (ml plasma/24 hr)
1	80	96	C	$1.4 \pm 0.075^+$	1.9 ± 0.015	75.9 ± 7.9
			E	0.9 ± 0.087	2.0 ± 0.021	45.9 ± 7.9
				< 0.001	NS	$p < 0.025$
2	80	96	C	1.4 ± 0.06	2.0 ± 0.6	76.0 ± 4.9
			E	1.1 ± 0.05	3.0 ± 0.2	46.5 ± 5.6
				< 0.005	< 0.01	< 0.001
3	40	96	C	1.5 ± 0.05	3.0 ± 0.4	62.2 ± 3.1
			E	1.3 ± 0.08	3.0 ± 0.5	49.6 ± 2.1
				< 0.05	NS	< 0.05
4	40	96	C	0.98 ± 0.02	2.7 ± 0.1	34.8 ± 1.6
			E	0.92 ± 0.03	3.5 ± 0.2	26.4 ± 2.3
				NS	< 0.005	< 0.01
5	40	110	C	1.63 ± 0.09	5.5 ± 0.3	25.9 ± 1.7
			E	1.42 ± 0.32	6.4 ± 0.2	24.1 ± 1.8
				NS	< 0.025	NS

+ Mean \pm se C - controls; E - O_2 -exposed

TABLE 4

Thyroxine turnover studies in rats exposed to hyperoxia

Expt	Group	k	TDS (ml)	Metabolic Clearance	Urinary Clearance	Fecal Clearance	Serum T4 Concentration $\mu\text{g}/100\text{ ml}$
(ml plasma / 24 hr)							
100% O ₂	C	0.44 \pm 0.037 ⁺	15.3 \pm 0.73	6.71 \pm 0.49	3.9 \pm 0.30	2.76 \pm 0.26	4.7 \pm 0.48
3 days	E	0.43 \pm 0.03	16.6 \pm 1.32	7.05 \pm 0.42	3.2 \pm 0.38	3.90 \pm 0.24	3.4 \pm 0.32
		NS	NS	NS	NS	< 0.01	< 0.05
100% O ₂	C	0.40 \pm 0.03	13.2 \pm 0.79	5.21 \pm 0.22	3.24 \pm 0.19	1.96 \pm 0.18	4.9 \pm 0.33
3 days	E	0.54 \pm 0.02	9.38 \pm 0.29	5.04 \pm 0.11	2.61 \pm 0.13	2.43 \pm 0.09	2.8 \pm 0.20
		< 0.005	< 0.005	NS	< 0.025	< 0.05	< 0.001
80% O ₂	C	0.48 \pm 0.02	18.3 \pm 1.28	8.74 \pm 0.53	4.99 \pm 0.23	3.75 \pm 0.36	5.7 \pm 0.32
4 days	E	0.45 \pm 0.01	19.7 \pm 1.14	8.99 \pm 0.55	5.77 \pm 0.32	3.19 \pm 0.24	4.3 \pm 0.27
		NS	NS	NS	NS	NS	< 0.01
80% O ₂	C	0.45 \pm 0.006	13.7 \pm 0.39	6.16 \pm 0.13	3.8 \pm 0.13	2.36 \pm 0.13	3.95 \pm 0.27
5.5 days	E	0.48 \pm 0.026	12.3 \pm 0.42	5.92 \pm 0.24	3.5 \pm 0.35	2.24 \pm 0.19	2.65 \pm 0.24
		NS	< 0.05	NS	NS	NS	< 0.005
80% O ₂	C	0.45 \pm 0.016	12.62 \pm 0.79	5.66 \pm 0.20	4.61 \pm .36	1.62 \pm 0.13	3.28 \pm 0.29
7 days	E	0.42 \pm 0.023	12.13 \pm 0.34	5.06 \pm 0.20	3.26 \pm .36	2.79 \pm 0.13	3.15 \pm 0.24
		NS	NS	< 0.05	< 0.025	< 0.01	NS

Mean \pm se
fractional disappearance rate
S: Total distribution space

C - controls; E - O₂-exposed

TABLE 5

Effect of Vitamin E on T_4 metabolism in rats exposed to hyperoxia

Expt	O ₂ exposure (hr)	Group	Urinary I^- ($\mu\text{g} \equiv T_4$)	Serum T_4 ($\mu\text{g}/100 \text{ ml}$)	Urinary clearance of T_4 (ml plasma/24 hr)
1	24	Cont.	$0.65 \pm 0.04^+$	1.6 ± 0.09	93.0 ± 12.8
		Cont + O ₂	0.46 ± 0.04	1.7 ± 0.07	90.0 ± 11.6
			NS	NS	NS
		Vit E	0.63 ± 0.021	1.7 ± 0.05	80.5 ± 6.5
2	48	Vit.E + O ₂	0.70 ± 0.11	1.8 ± 0.07	95.3 ± 20.1
			NS	NS	NS
		Cont.	0.87 ± 0.058	0.7 ± 0.04	109.7 ± 7.85
		Cont + O ₂	0.61 ± 0.035	0.8 ± 0.04	68.1 ± 4.8
			< 0.001	NS	< 0.001
		Vit E	0.848 ± 0.11	0.8 ± 0.037	90.6 ± 14.3
		Vit E + O ₂	0.841 ± 0.05	1.2 ± 0.18	79.7 ± 13.0
			NS	NS	NS

+ Mean \pm se

TABLE 6

Effect of hyperoxia in vivo on deiodinating activity in isolated tissue preparations in vitro

Expt.	Duration of exposure (hr)	Tissue	Deiodinating activity (% T ₄ deiodinated)		p value
			Control	Exptl.	
1	48	Muscle	25.2 ± 2.20 ⁺	18.4 ± 0.66	< 0.025
	48	Liver	04.2 ± 3.20	67.1 ± 4.76	NS
	48	Kidney	32.2 ± 3.1	30.7 ± 4.2	NS
2	48	Muscle	30.5 ± 3.18	18.6 ± 1.8	< 0.01
	48	Liver	63.3 ± 9.54	68.2 ± 1.53	NS

+ Mean ± se. Deiodinating activity was determined in homogenates, 1:100 wt/vol prepared in KRPG pH 7.0. ¹²⁵I-T₄, 10⁻⁹ M was employed. Incubations were carried out for 1 and 2 hr. (2 hr value shown here) under 95% O₂: 5% CO₂.

Table 7

Effect of hyperoxia or serum T₄-binding activity

Expt.	Duration of Exposure (hr)	Binding activity		
		(% total control	125-I-T ₄ in supernatant) Expt.	P value
1	48	56.6 ± 1.67 ⁺	46.8 ± 1.6	< 0.001
2	48	40.9 ± 1.20	32.5 ± 1.35	< 0.005
⁺ Mean ± se				

TABLE 8

Effect of hyperoxia on oxygen consumption in rats

<u>Treatment</u>	<u>Oxygen uptake ml O₂/min/rat</u>
Air	5.43 ± 0.24
Oxygen (100% 48 hr)	3.98 ± 0.15
p value	< 0.001

Mean ± S.E.

TABLE 9

Effect of acute cold exposure on peripheral metabolism of ^{125}I -thyroxine

Day ⁺	Rats [‡]	Weight (g)	Serum T ₄ ($\mu\text{g}/100\text{ ml}$)	T ₄ excreted in urine as I ($\mu\text{g T}_4/24\text{ hr}$)	Urinary ^{125}I (% dose)	T ₄ clearance values(ml plasma/24 hr)	Deiodination	Fecal [‡]
						Total		
1	Control (8)	308 \pm 8.6	4.19 \pm 0.5	2.68 \pm 0.12	53 \pm 2.4	55 \pm 3.3	30 \pm 2.7	25 \pm 1.7
	Cold (8)	272 7.6	8.29 0.6	2.46 0.15	49 3.1	62 5.2	31 3.3	31 2.9
	p value	< 0.01	NS	NS	NS	NS	NS	NS
2	Control (8)	314 7.8	9.89 0.4	2.06 0.08	41 1.7	51 2.3	21 1.1	30 1.6
	Cold (8)	275 7.3	8.84 0.6	1.50 0.12	30 2.3	58 4.1	17 1.7	40 3.3
	p value	< 0.005	NS	< 0.005	< 0.005	NS	NS	< 0.025
3	Control (8)	313 7.5	9.05 0.5	2.21 0.11	44 2.2	56 3.2	24 1.6	31 2.4
	Cold (8)	272 8.2	9.08 0.4	1.87 0.15	37 2.9	55 2.3	21 2.4	34 1.1
	p value	0.005	NS	NS	NS	NS	NS	NS
4	Control (8)	310 7.6	8.20 0.6	2.98 0.13	59 2.5	62 4.3	37 2.4	25 2.8
	Cold (8)	257 7.4	7.60 0.4	2.48 0.13	49 2.6	66 3.2	33 2.4	34 2.9
	p value	0.001	NS	0.025	0.025	NS	NS	NS

* Values shown are the mean \pm SE. + Day refers to end of particular 24 hour collection. ‡ Numbers in parentheses indicate number of rats/group. ‡ Calculated as the difference between total and deiodinative clearance.

TABLE 10

Thyroxine turnover studies in rats acutely exposed to 4° C

Expt.	Group	k	TDS (ml)	Metabolic Clearance	T _{1/2}	¹²⁵ I in urine (% dose/30 hr)
1	Controls	0.65 ± 0.04*	18.8 ± 1.25	12.1 ± 8.2	11.4 ± 0.9	6.1 ± 0.38
	Cold-exposed	0.93 ± 0.08	20.2 ± 1.09	18.18 ± 9.6	7.5 ± 0.1	32.7 ± 2.20
	p value	< 0.001	NS	< 0.001	< 0.005	< 0.001
2	Controls	0.48 ± 0.01	42.0 ± 1.17	20.08	12.6 ± 0.7	10.2 ± 0.78
	Cold-exposed	0.49	64.7 ± 4.5	30.89	8.2 ± 0.6	25.7 ± 1.78
		NS	< 0.001	< 0.001	< 0.005	< 0.001

* Mean ± se

k = fractional disappearance rate

TDS = Total distribution space

T_{1/2} = Biological half-life of T₄

TABLE 11

Effect of PTU on survival of rats at a simulated altitude of 20,000 feet

% Survival for 48 hr.

Group	Treatment	Expt 1	Expt 2	Expt 3
I	T ₄	30	50	20
II	T ₄ + PTU	80	90	80

PTU : 2 µg/9 diet
T₄ : 2 µg/100 g BW/day

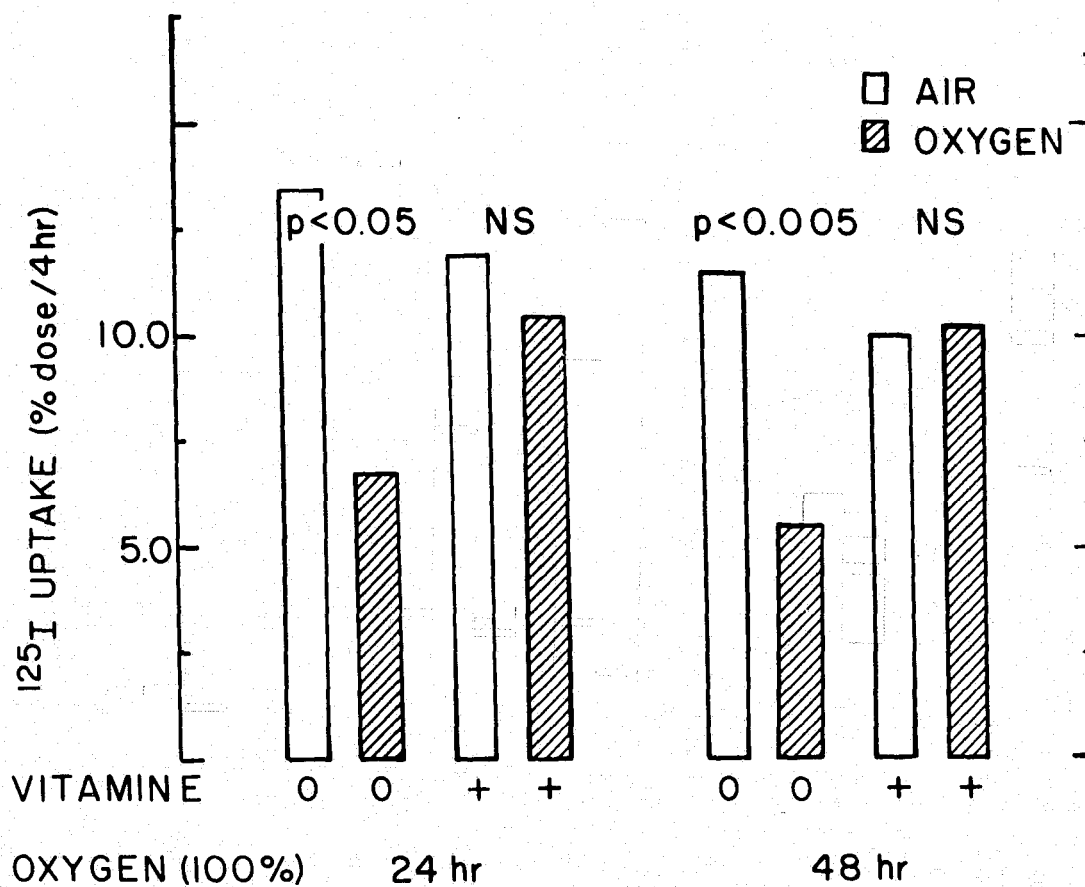


Figure 1. Effect of vitamin E on the oxygen-induced depression of thyroid function in mice. Vitamin E was given in the diet for three days prior to oxygen exposure (20 mg α -tocopheryl sulphate/10 g food) and was also injected on each of the days of exposure (20 mg Vitamin E in 0.25 ml sesame seed oil/rat).

GROUP	TREATMENT	% SURVIVAL FOR 3 DAYS		
		EXPT 1	EXPT 2	EXPT 3
I	-	90	100	100
II	PTU	16.7	0	0
III	T ₄	100	100	100
IV	PTU + T ₄	70	100	87.5

PTU: 2 mg /g diet

T₄: 2 μ g / 100g BW / day

Figure 2. The effect of PTU and T₄ on the survival of rats exposed to 4° C.

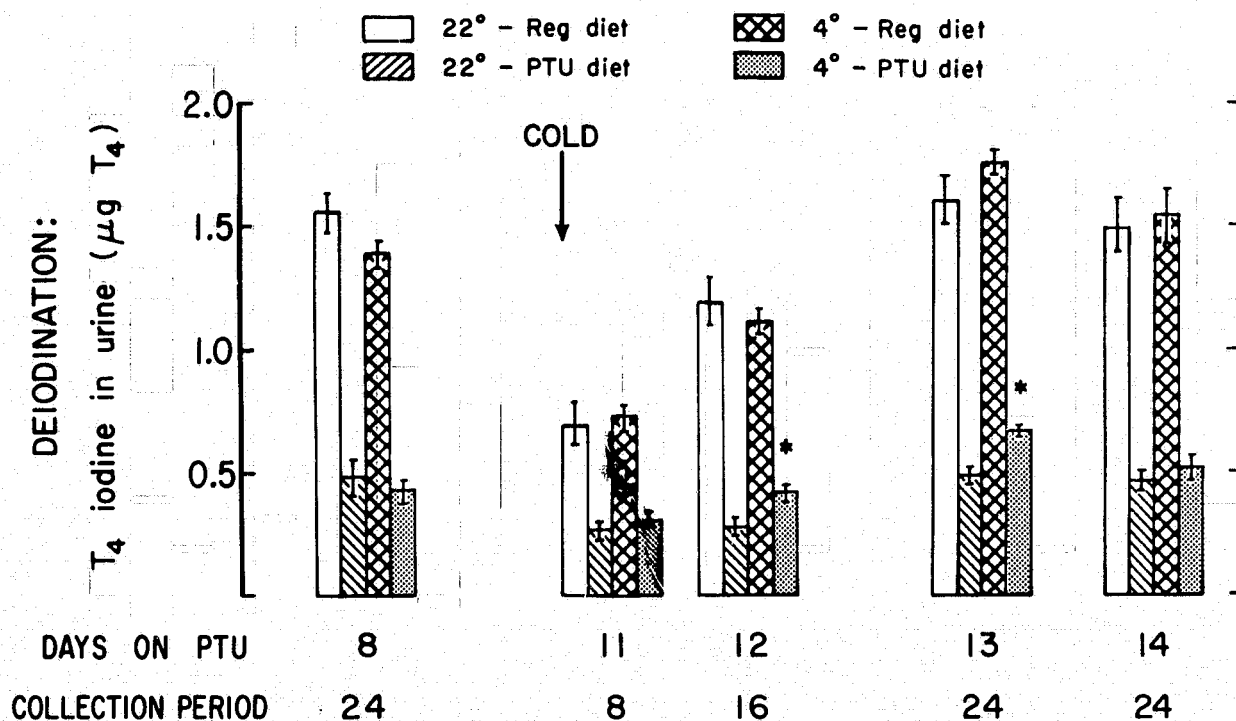


Figure 3: The effects of PTU and cold exposure on the rate of deiodination of T_4 in rats. Approximate $^{125}I/^{127}I$ equilibrium was achieved by administration of $^{125}I-T_4$, as a daily sc injection, $2 \mu g/100 g$ BW, for 14 days prior to the start of the experiment and continued for the 14 days of the experiment. Thyroidal uptake of ^{125}I was prevented with $NaClO_4$ (1% in drinking water).

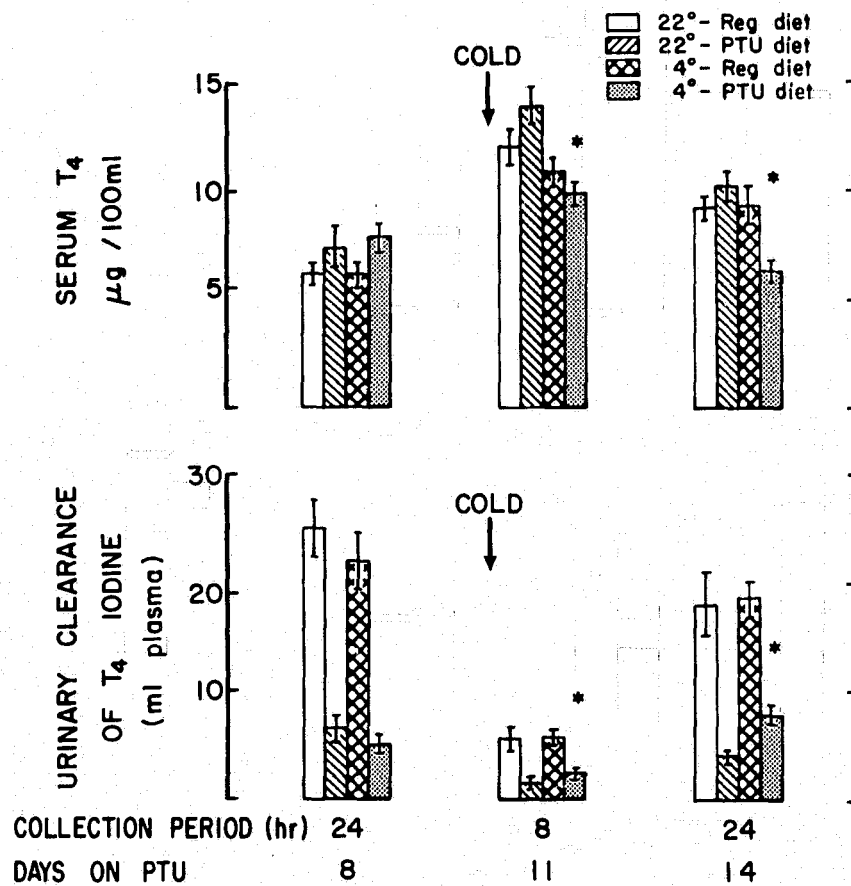


Figure 4: The effects of PTU and cold exposure on serum T_4 concentration and clearance of T_4 iodine in urine. Experimental details as in Fig. 3.

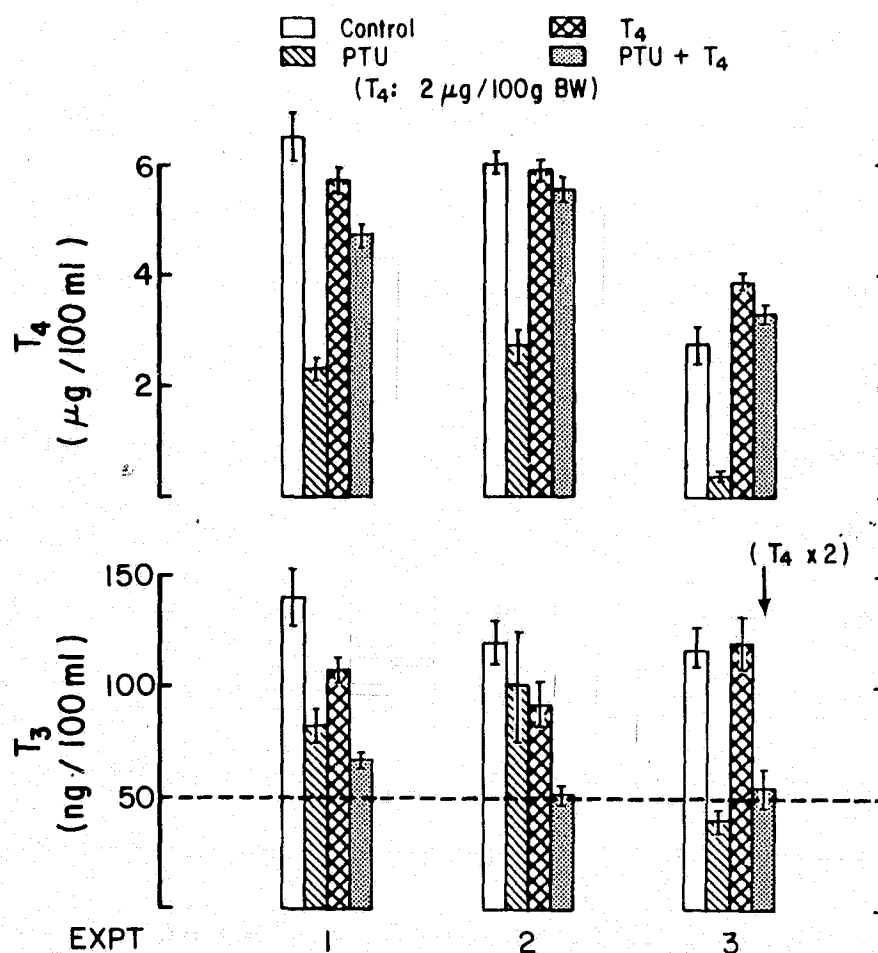


Figure 5: Serum T₄ and T₃ levels in normal and PTU-treated rats, with and without supplemental T₄, following exposure to 4° C for 3 days. In experiments 1 and 2, PTU (2 mg/ g food) was given for 2 weeks; in experiment 3 it was given for 4 weeks. T₄ was given for the last 4 days prior to cold exposure.